298. *The Carbonyl Constituents of Eucalyptus Oils. Part II. The Seasonal Variation of* E. cneorifolia *Oil.*

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The oils obtained by monthly distillations of (*a*) old leaf and (*b*) young leaf and growing tips of *E. cneorifolia* were regularly examined with the object of searching for evidence of phytochemical synthesis of the components; and also of seeing if cryptal occurred in the oil at any season of growth.

The yield of young leaf oil increases during the period of active growth, and side by side with this a fall in the density and a rise in the *l*-rotation of the oil are observed, these changes being correlated with the increase in the terpene content of the oil. No marked similar change is found in the oils from old leaf.

The terpenes are found to contain considerable quantities of *l*-β-phellandrene during the flush period, and the biogenetic relationship *l*-β-phellandrene, *l*-phellandral, *l*-4-*iso*propyl-Δ^2-*cyclo*hexen-l-one is suggested. *l*-α-Phellandrene and cymene are also present in these oils, the cymene content decreasing in the winter months.

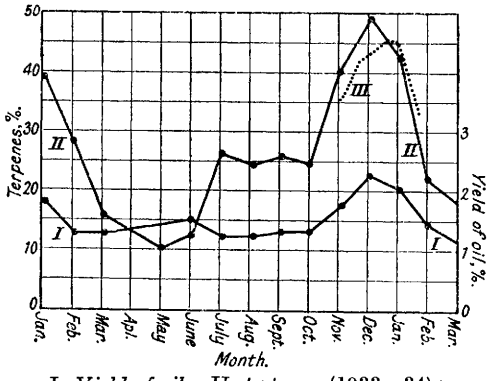
No cryptal was detected during the examination of upwards of fifty samples of the oils.

The regular examination of the oil of *E. cneorifolia* was first undertaken in the hope that seasonal variation in the components would afford some evidence of phytochemical change on which biogenetic relationships might be based: and with the later object of also ascertaining if the aldehyde cryptal, previously reported, occurred in the oil at any period of the year. It now appears (this vol., p. 986) that the existence of cryptal was assumed in error, and the present examination of upwards of fifty samples of *E. cneorifolia* oil, which has extended over several years, has failed to disclose any proof of its existence.

The biogenesis of the constituents of essential oils has been discussed by, amongst others, Read (*J. Soc. Chem. Ind.*, 1929, **48**, 786; *Chem. Reviews*, 1930, **7**, 41) and Hall (*ibid.*, 1933, **13**, 479). The suggestions made have generally been based on the mutual occurrence of components, one of which, such as geraniol, may readily be shown to give rise to the formation of the others by a series of simple changes involving cyclisation, dehydration, hydration, and intramolecular change. The work now described gives results on which, we suggest, biogenetic relationships may more confidently be based.

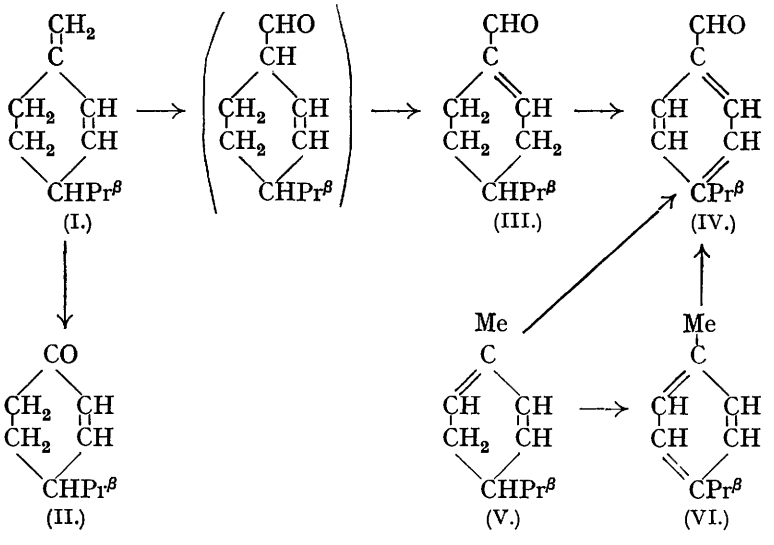
The examination of *E. cneorifolia* oil obtained by monthly distillations of old leaf showed no marked change in the nature of the components or in their amounts throughout the year. On the other hand, wide fluctuations were found in the oil obtained from the young leaf and growing tips (apart from minor variations inseparable from the distillation of

comparatively small quantities of leaf on an experimental scale). The yield of oil (see fig.) increases greatly in the late spring and early summer months, which is the period of active growth: and the oil has then an exceptionally large hydrocarbon content. Despite the fact that *E. cneorifolia* oil is regarded as one from which phellandrenes are absent, a large proportion of such hydrocarbons was actually present in the oil in the flush period, the summer oils of young leaf readily responding to the phellandrene nitrosite test: and at this period the aldehyde content was correspondingly low.



I, *Yield of oil;* II, *terpenes* (1933—34); III, *terpenes* (1934—35).

In view of the large amounts of phellandrene present in the young leaf oils at the period of free growth, their availability as initial material for phytochemical synthesis seems to be clearly established. As the season advances the phellandrene content of the oil shows a marked decrease, and in view of the fact that β-phellandrene (I) is readily converted into 4-*iso*propyl-Δ^2-*cyclo*hexen-1-one (II) by aerial oxidation in sunlight, and also yields phellandral (III) by permanganate oxidation under controlled conditions (Wallach, *Annalen*, 1905, **340**, 1; **343**, 35), it appears valid to assume that these are formed in the eucalyptus oils by phytochemical synthesis from this hydrocarbon (see Galloway, Dewar, and Read, J., 1936, 1595).



In support of this it has been observed that the aldehyde content of some eucalyptus oils increases on storage in partly filled bottles: and the hydrocarbon fractions of the young oils at the flush period give, after treatment with air or oxygen in ultra-violet light, aldehyde values as high as 15%; and the ketone *l*-4-*iso*propyl-Δ^2-*cyclo*hexen-1-one has been isolated from such reaction products.

The phellandrenes in the oils have been systematically examined, and the work will be described in a further communication. Both *l*-α- and *l*-β-phellandrene are present, and the stereochemical biogenetic relationship *l*-β-phellandrene, *l*-phellandral, *l*-ketone is suggested. An interesting confirmation of this view is found in the case of water-fennel oil, in which the relationship *d*-β-phellandrene, *d*-phellandral, *d*-ketone has been established (see following paper).

Cuminal (IV) may be formed in the oil by dehydrogenation of phellandral, and this

would appear to be the most direct way to account for its occurrence. On the other hand, it may be derived from α-phellandrene (V) (Penfold and Simsonsen, J., 1930, 403) or alternatively from cymene (VI).

Borgwardt and Schwenk (*J. Amer. Chem. Soc.*, 1934, **56**, 1185) have shown that on treatment with selenious acid α-phellandrene is oxidised to cuminal; and Stephens (*ibid.*, 1926, **48**, 1824) has also isolated and identified this aldehyde as an oxidation product of cymene by gaseous oxygen at 85°. About 19% of cymene is present in the young leaf oil in September, but this decreases to some 3—4% as the season advances. It has been obtained from α-phellandrene, and may be so derived in eucalyptus oils; and the fact that the cymene content of the summer oils is low, and also that their ratio of phellandral to cuminal is much lower than in the winter oils, may not be without significance in connection with the latter view of cuminal formation.

Experimental.

Distillations.—The distillations were carried out at American River, Kangaroo Island, in a direct-fired, experimental, 45-gallon still coupled to a water-cooled, copper worm-condenser. The still was charged with leaf (100 lbs.) and water (12 gallons), and the distillations continued over a period of 6—7 hours, during which the oil and approximately 7 gallons of aqueous distillate collected. The distillation was thus pushed further than is common in commercial practice, the resultant oils being consequently richer in aldehydes. The crude oils were analysed without further rectification, cineole being determined by the *o*-cresol method; alcohols, calculated as $C_{10}H_{18}O$, by difference between the ester value of the oil and the ester value after acetylation; aldehydes by the hydroxylamine method; and terpenes by difference. Typical results with growing tips and terminal branchlets are set out in Table I and shown in the figure, percentages being by weight. The leaf in all cases was cut in the same scrub area.

Table I.

Crude oils from young leaf.

Date.	Yield, %.	$d^{15.5°}_{15.5°}$.	$[\alpha]_D$.	Alcohols, %.	Aldehydes, %.	Cineole, %.	Terpenes, %.
2/1/33	1·80	0·8944	−22·10°	9·7	12·2	39·0	39·1
6/2/33	1·28	0·9138	−11·48	9·7	18·7	43·0	28·6
6/3/33	1·28	0·9227	−10·95	8·5	20·1	56·0	15·4
1/5/33	1·40	0·9232	−14·55	12·6	20·3	56·8	10·3
5/6/33	1·49	0·9248	−12·93	11·6	21·5	54·4	12·5
2/7/33	1·19	0·9237	−14·91	6·7	17·5	49·4	26·4
7/8/33	1·19	0·9217	−17·03	10·9	17·9	46·4	24·8
4/9/33	1·28	0·9213	−14·40	6·9	20·6	46·0	26·5
2/10/33	1·30	0·9109	−17·35	8·5	17·3	49·8	24·4
6/11/33	1·74	0·8906	−24·18	8·3	6·8	44·2	40·7
4/12/33	2·24	0·8795	−31·27	7·8	6·8	36·0	49·4
2/1/34	2·02	0·8927	−23·70	7·3	12·0	38·4	42·3
5/2/34	1·44	0·9183	−14·27	10·3	18·7	49·0	22·0
5/3/34	1·19	0·9206	−13·32	8·9	20·2	53·0	17·9
4/11/34	1·73	0·8961	−22·79	7·9	10·1	46·4	35·6
19/11/34	2·02	0·8940	−24·05	6·5	8·9	42·6	42·0
2/12/34	2·00	0·8926	−23·64	5·3	8·5	42·8	43·4
17/12/34	2·09	0·8876	−27·24	5·85	7·9	40·8	45·45
1/1/35	1·94	0·8910	−25·68	5·85	9·15	39·8	45·2
22/1/35	1·83	0·9031	−18·82	5·1	14·7	47·0	33·2

Apart from minor fluctuations which are to be expected in small-scale work, the results show clearly a definite increase in the yield of oil during the period of active growth. The oils at this season are characterised by a rise in the lævo-rotation and a fall in the density, which are doubtless correlated with the marked increase in the terpene content.

Side by side with the separation of oils from the growing tips, a series of similar distillations on old leaf was carried out. The results in this case showed only minor fluctuations and no marked increase in either rotation or terpene content was recorded.

To supplement the work, arrangements were made with a commercial distiller in an adjoining district for the monthly distillation of 1-ton lots of the leaf. Such leaf was naturally not subject to selection and included the young tips during the months of active growth. The rotation of the oils at this period rose from an average value of some − 5° to approximately − 9°. There was also a 5% increase in the terpene content, which rose to about 19%.

Isolation of Aldehydes.—The oils from the small-scale distillations were treated directly for the removal of carbonyl compounds by successive shaking with neutral sodium sulphite solution (5 vols.; 35% $Na_2SO_3,7H_2O$) and saturated sodium bisulphite solution (2 vols.). The neutral sulphite action is continued until phenolphthalein is no longer coloured pink, the alkali liberated being periodically neutralised by 10% sulphuric acid during treatment. This method of prior removal of the ketone is found to be much better than direct treatment with bisulphite, for in the latter case with oils rich in the ketone, considerable quantities of this are found to remain as a solid derivative in the bisulphite cake, and are subsequently isolated with the phellandral on treatment with boiling bisulphite solution in the usual purification process. The oil unabsorbed on treatment with neutral sulphite is separated and shaken for 8 hours with the bisulphite solution, the sodium sulphite extract being meanwhile decomposed with 40% sodium hydroxide, after washing with ether. The product so obtained consisted essentially of the ketone *l*-4-*iso*propyl-Δ^2-*cyclo*hexen-1-one, containing traces of phellandral. The unabsorbed oil filtered from the bisulphite cake was reserved for further treatment, and the cake itself was well washed with alcohol–chloroform–ether to remove all traces of oil. The bisulphite solution yielded a small quantity of oil on treatment with 40% sodium hydroxide solution; and this was found to consist of *l*-phellandral and some ketone. The bisulphite cake, decomposed by 5% sodium carbonate solution, gave a mixture of cuminal and *l*-phellandral, and a further small quantity of *l*-phellandral with traces of cuminal was recovered after this treatment by the addition of sodium hydroxide. The purification of cuminal and phellandral was carried out in the usual way by boiling with bisulphite solution. The ketone and the aldehydes were identified in all cases by comparison of their derivatives (semicarbazones, *p*-nitro- and 2 : 4-dinitrophenylhydrazones) with authentic specimens. Only the ketone, cuminal, and *l*-phellandral were found in the oils. The ketone appears to be present in larger amounts than cuminal or phellandral, the quantities isolated constituting 40—55% of the total estimated amount of carbonyl compounds. It is difficult to determine with any great accuracy the relative amounts of cuminal and phellandral present, but as the rotation of the mixed aldehydes obtained from the bisulphite cake rose from — 6·7° in the December oil, or — 11·5° in November, to some — 40° in the winter oils, it is evident that cuminal preponderates in the oils of the flush period, but the proportion of phellandral increases in the winter months.

As no marked variation was found from month to month in the case of the commercial oils, the yields from November to February were mixed and treated as summer oil bulk, the remaining oils being mixed for winter oil bulk. As the cineole content was high (70—74%) and the aldehyde content low (7—9%), the separate bulks were refrigerated; and in this way upwards of one-third of the oil was removed as cineole (96% pure). The residues (6·5 gallons summer oil, $d^{15\cdot5°}_{15\cdot5°}$ 0·9135, $[\alpha]_D$ — 7·49°, aldehydes 11·1%, cineole 59·0%; 12 gallons winter oil, $d^{15\cdot5°}_{15\cdot5°}$ 0·9206, $[\alpha]_D$ — 6·41°, aldehydes 12·7%, cineole 61·6%) were distilled with live steam to concentrate the aldehydes in the later fractions. The aldehydes and the ketone were isolated from the fractions by the procedure already described, with the following typical results.

Fraction.	Vol., c.c.	$d^{15\cdot5°}_{15\cdot5°}$.	$[\alpha]_D$.	Ketone, g.	Cuminal, g.	Phellandral, g.
Winter, 6	2150	0·9426	—23·9°	337	130	67
,, 7	2170	0·9583	—33·45	422	215	65
Summer, 5	2200	0·9290	—15·33	315	100	4
,, 6	1420	0·9539	—31·68	301	221	33

In all cases the oils were carefully examined for aldehydes other than the three main carbonyl compounds, but with negative results. It will be seen that the phellandral obtained amounts to some 34% of the total cuminal + phellandral isolated in winter oil fraction 6; and 23% in fraction 7. The values in the corresponding fractions of the summer oil were 4 and 13% respectively.

Hydrocarbons and Terpenes.—The oils remaining after the isolation of the ketone and the aldehydes were shaken, after thorough washing to remove bisulphite, with 1·5 volumes of 50% resorcinol solution to remove cineole. The semi-solid cake obtained on seeding with the cineole–resorcinol compound was filtered off with suction, and repeatedly washed with the aqueous portion of the filtrate to remove adhering oil. The resorcinol treatment was repeated three or four times (depending on the amount of cineole), and the oil finally distilled under reduced pressure after thorough washing and drying. The distillates were aldehyde-free and contained no appreciable quantities of cineole or alcohols. Representative results are summarised in Table II.

The marked difference in the nature of the terpenes present in the oils during the flush

TABLE II.

Hydrocarbon and terpenes of young leaf oils.

Oil.	Fraction.	Vol., c.c.	$d_{15\cdot5^\circ/15\cdot5^\circ}^{15\cdot5^\circ}$.	$[\alpha]_D^{20^\circ}$.	Oil.	Fraction.	Vol., c.c.	$d_{15\cdot5^\circ/15\cdot5^\circ}^{15\cdot5^\circ}$.	$[\alpha]_D^{20^\circ}$.
Jan.	(1) 54°/10 mm.	30	0·852	−30·8°	Oct.	(1) 48°/8 mm.	7·5	—	−18·3°*
	(2) 52/8 mm.	60	0·854	−33·3		(2) 48/6 mm.	36	0·857	−22·8
	(3) to 60/8 mm.	55	0·861	−26·8		(3) to 52/6 mm.	39	0·865	−17·3
Feb.	(1) 45—47/5 mm.	36	0·855	− 0·75	Nov.	(1) 52/8 mm.	17	0·849	−44·2
	(2) 47/5 mm.	24	0·861	− 1·8		(2) 48/5 mm.	77	0·848	−45·6
	to 105/5 mm.	2	—	—		(3) to 58/5 mm.	70	0·853	−37·2
May	(1) 50/8 mm.	5	—	+ 1·12*	Dec.	(1) 62—65/9 mm.	45	0·846	−46·8
	(2) 52/6 mm.	30	0·867	+ 0·66		(2) 59—61/6 mm.	75	0·847	−51·4
	(3) 52/6 mm.	22	0·875	+ 0·07		(3) 62/7 mm.	45	0·848	−46·1
Sept.	(1) 65/11 mm.	9	—	+ 0·08*		(4) to 60/4 mm.	45	0·854	−33·4
	(2) 61—62/6 mm.	30	0·867	+ 0·07					
	(3) 62—63/5 mm.	22	0·885	− 0·74					

* Values are those of α_D.

period and those occurring in the other months is shown by the high *l*-rotations of the former fractions; and the high *l*-rotations of the crude oils themselves are therefore evidently due to terpenes. All the terpene fractions gave a nitrosite test, but phellandrene was found to be present in considerable amounts in those fractions distilled from the January, October, November, and December oils; and the respective crude oils themselves gave a phellandrene nitrosite test. Crude oils distilled during the other months did not give a nitrosite at laboratory temperatures, although the July, August, and September oils responded weakly to the test at − 10°. The presence of *l*-β-phellandrene is shown by oxidation of rich fractions by oxygen in ultraviolet light. The aldehyde estimation after irradiation gave a value of some 15% in cases examined, and *l*-4-*iso*propyl-Δ^2-*cyclo*hexenone was isolated by neutral sodium sulphite and identified by its derivatives. *l*-α-Phellandrene is also present, and the results of a detailed examination of the nitrosites will be communicated later.

The presence of pinene in the terpene fractions was indicated by colour development during the phellandrene test. The fractions from the May to September oils gave the most pronounced colour tests, and although attempts to form a nitrosochloride failed, a colorimetric estimation showed a pinene content of some 3%.

All attempts to prepare the characteristic limonene tetrabromide were unsuccessful, although the numerous modifications mentioned in the literature were tried.

Cymene.—Cymene has been detected in a number of the oils from the growing tips. Treatment of the terpene fractions with potassium permanganate in the cold effectively removes the terpenes. In a typical experiment, 20 c.c. of fraction 2 of September oil were shaken with 4% potassium permanganate solution in the cold, finely powdered permanganate being added as oxidation proceeded, and until a permanent pink remained for 24 hours (20 g. required). The residual oil (16 c.c.; α_D 0·04°; $d_{15\cdot5^\circ/15\cdot5^\circ}^{15\cdot5^\circ}$ 0·874) was recovered by steam distillation. By admixture with pure cineole, the oil was found to contain 10·6% of cineole, by the *o*-cresol method. Oxidation by hot permanganate (Wallach, *Annalen*, 1891, **264**, 1) gave an acid which, after recrystallisation, had m. p. 158° and is evidently *p*-hydroxy*iso*propylbenzoic acid (Found, in barium salt: Ba, 27·3. Calc.: Ba, 27·7%). An estimate of the amount of cymene present in the oil follows from the isolation from 20 c.c. of terpene fraction (*d* 0·867) of 16 c.c. of cymene (*d* 0·874) containing 10·6% of cineole. The terpene content of the crude oil is a maximum of 26·5%, whence the cymene percentage in the crude oil is estimated as 26·5 × 12·5/17·34 = 19·0% by weight. This is a maximum value, as some phellandrene is removed by the resorcinol treatment, leaving the terpene fraction correspondingly richer in cymene. Cymene was similarly isolated as an inactive oil from the January, February, September, November, and December terpene fractions, and similar estimates gave the respective values of 9·5, 13·7, 19·0, 3·0, 4·0%.

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